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Effects of Rhizobium Inoculation and Phosphorus Fertilizer rates on Nitrogen Fixation and Nutrient up take of Chickpea (*Cicer arietinum* L.) at Goro, Bale Zone, Oromia Regional State.

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ABSTRACT

Chickpea response to two varieties of chickpea (Arerti and Habr), two Rhizobial inoculants (EAL 018 and EAL 029) and five P rate (0, 15, 30, 45 and 60 kg P2O5 ha-1) were studied in split plot arrangements. Main plot consisted of varieties whereas P2O5 levels and Rhizobium strains were kept in subplot. The experiment was laid out at Goro, Bale Zone, Oromia Regional State during 2015/16 with the objective to determine the effectiveness of Rhizobium strains and phosphorus fertilizer application on two varieties of chickpea (Arerti and Habru).The nodulation rating, nodule volume and color were not significantly affected due to varieties. However, significant variation (P <0.05) was observed in number of nodule per plant (NNPP) and nodule dry weight (NDW) between varieties of chick pea, maximum NNPP and NDW was obtained from Arerti variety. All the nodulation parameters were significantly (P<0.05) affected due to rhizobium inoculation and P2O5 except the nodule color. Maximum nodulation parameters were recorded at 45 and 60 kg P2O5 ha-1and inoculation by EAL 029 strain except the nodule color. Significant interaction effect was observed between variety and Rhizobium inoculation for nodule rate (NR), NDW between variety and P2O5, rhizobium and P2O5 and also interaction effect between varieties, Rhizobium and P2O5 on NR and NDW. The N and P uptake at mid flowering, %Ndfa, grain, straw and total N and P content and crude protein content were significantly affected by variety, rhizobium inoculation and application of P2O5. Maximum response was obtained from EAL 029 strain inoculations and application of 45 kg P2O5 ha-1 for N uptake, seed N and P uptake were maximum at 45and 60 kg P2O5 ha-1 respectively. The current investigation indicated that Arerti variety inoculation with Rhizobium strain EAL 029 along with application of P2O5 at rate of 45 kg ha-1 found to be appropriate for chickpea production in the study area. The current investigation indicated that the use of P2O5 with Rhizobium inoculation as a nutrient management strategy could increase chick pea production. Since the experiment was conducted only for one year, we suggest the results to be verified with more varieties of chickpea and rhizobium strain under the same agro-climatic conditions.

INTRODUCTION

Chickpea (*Cicer arietinum* L*.)* is an important leguminous crop with high nutritive value and source of protein. It is originated in southeastern Turkey (Redden and Berger, 2007).The crop is adapted to cool semi-arid areas of the tropics, sub-tropics as well as the temperate areas. It is the third most important crop in volume of production after Faba bean and haricot bean, in Ethiopia (CSA, 2015). Legume crops such as chickpea, faba bean and field pea were the potential crops which can be grown in rotation to break the mono-culture cropping practice in Bale area. National average yield of chickpea in Ethiopia is 1.9 t ha^{-1} which is far below the potential yield of 4.5 t ha-1 (CSA, 2015). In south eastern Ethiopia, the average of 1.18 t ha^{-1} is even below the national average. The gap between actual and potential yield is the result of limited application of P fertilizer and rhizobium inoculation for chickpea production in the study areas.

The amount of P_2O_5 rate and effective type of rhizobium strain were not identified for the Goro area. Most tropical soils are deficient in available phosphorus and in terms of appropriate and effective strains that are capable of fixing nitrogen. There is a need to identify appropriate *rhizobium* strain which will enhances nitrogen fixation attributes and yield of chickpea under different phosphorus levels. However, the recommended rate of fertilizer might vary according to crop type (variety), location, soil type, etc. Therefore; this research was initiated to study the effect of *rhizobium* inoculants and phosphorus fertlizer rates on, nodulation, nitrogen fixation and nutrient uptrake of chickpea varieties

MATERIALS AND METHODS

Description of the Study Area

The experiment was conducted at Goro mid altitude of Bale, Oromia Regional State, and South Eastern Ethiopia during the main cropping season of 2015/16. Goro is located 30 km from Sinana Agricultural Research Center, 60 km from Bale Robe and 490 km from Addis Ababa. The site is situated at $6^0$59' N latitude and 40^0 29'E longitude and elevation of 1771 meters above sea level. The area has bimodal rainfall patterns. Based on this there are two separate crop growing seasons locally called '*bona'* and '*gana'.* The main season *bona* extends from August to December and *gana* from March to May. The area receives a mean annual rainfall of 500 mm, with annual mean maximum and minimum temperatures of 20^0C and 16 ^{0}C, respectively. The soils are predominantly vertic in properties.

Treatments and Experimental Design

The experiment was laid out as a split plot design where two chickpea varieties (Arerti and Habru) were obtained from Debrezeyit Agricultural Research Center (DZARC) was allocated on the main plot, while factorial combined five phosphorus fertilizer rates (0, 15, 30, 45, and 60 kg P_2O_5 ha^{-1}) and two *Rhizobium* strains (No inoculation, EAL 018, and EAL 029) was obtained from Menagesh Biotech PLC and Soil Microbiology Laboratory of National Soil Testing Center (NSTC), Addis Ababa, were assigned on the sub-plot in three replications. Treatments were assigned to each plot randomly. The total number of plots was 93 (90 for chickpea and 3 for wheat). The size of each plot was 4 m x 2.4 m (9.6 m^2) and the distance between the plots and blocks were 1m and 2m respectively. The distance between adjacent rows and plants was 0.3 m and 0.1 m respectively. Each plot was consisted 8 rows. At physiological maturity, plants from the central four rows of a net plot size of 1.2 x 4m (4.8m^2) were harvested and used for determining yield and yield components, while destructive sample were taken from the rest four rows for estimation of nodulation parameter. Reference crop wheat newly released variety Sanate was planted in the same area (4x2.4m) plots with 0.2m between rows and 0.05m between plants and consists of a total of 12 rows.

Experimental Procedures

Treatment application and field activities:

Before planting inoculation of the seeds was done using the dish as a container and sugar as adhesive material to stick the inoculums on the seeds. In oculation was done under the shade to avoid direct sun light. The inoculated seeds were kept in the shade for a few minutes to let them air dry before planting. Plots receiving non inoculants were planted before the others followed by those receiving inoculants in order to reduce the possibility of cross contaminations. Both *rhizobium* strains and P_2O_5 fertilizer were applied according to the treatments at the time of sowing. At planting 12 kg ha^{-1} of nitrogen fertilizer in the form of urea was applied to all plots as starter N in the chickpea treatments and the same amount was applied to wheat plots to keep the N balance. All weeds were removed by hand weeding and hoeing according to the locally recommended practice. Reference crop wheat (non nitrogen-fixer) was sown at the recommended seed rate of 125kg ha^{-1} and phosphorus fertilizer in the form of TSP (46 kg P_2O_5 ha^{-1}) was applied at planting. Harvesting was done when the leaves started to senesces and shedding and pods turned yellow.

Soil sampling, preparation and analysis: one composite soil samples (0-30cm depth) was taken randomly in a W-shaped pattern from the representative fifteen spots of the experimental field using an augur. Finally the samples were analyzed for the following parameters: particle size distribution, pH, organic carbon, cation exchange capacity (CEC), K, total nitrogen and available P from the representative bulk soil sample before planting. Similarly, a surface soil sample of the same depth (0-30cm) was collected just after harvest from each plot by taking samples from five spots and total N and P were analyzed for each treatment in all replications following the standard procedure after harvest.

RESULTS AND DISCUSSION

Table 1: Soil Physico-chemical Properties of the Experimental Site

Soil characteristics	Test result
pH (by 1:2:5 soil Water)	7.6
pH(Kcl)	6.89
Total N (N %)	0.17
CEC (cmol. (+) kg soil-1)	49.46
Av.P (ppm), Olsen	8.43
OC (%)	1.19
OM (%)	2.05
K+ (cmol. (+) kg soil-1)	2.83
Soil texture	
Clay	46%
Sand	20%
Silt	34%
Soil texture Class	Clay

Effect of phosphorus and *rhizobium* inoculation on Nodulation Parameters

Number of nodule per plant

Data concerning number of nodules per plant was significantly ($P<0.05$) affected due to varieties, rhizobium inoculation and phosphorus fertilizer rates. Mean data indicated that application of 60 and 45 kg P_2O_5 ha^{-1} significantly increased nodule number by 11.24 and 13.20% respectively in chickpea relative to the control. The increased in number of nodules per plant with P_2O_5 application could be because of P is required for plant growth, nodule formation and development, each process being vital for N_2 fixation. Phosphorus is known to initiate nodule formation as well as influence the efficiency of the rhizobium-legume symbiosis thereby enhancing nitrogen fixation (Haruna and Aliyu, 2011). *Rhizobium* inoculation with EAL 029 significantly increased number of nodules per plant by 7.19% than EAL018 and control presented in Table 2. This study revealed that inoculation significantly improved nodule number per plant as compared with uninoculated treatment. This might be because of inoculated bacteria strain had good nodulation inducing capacity over the native soil *rhizobium* population. Significance difference was observed between varieties in terms of nodule number per plant .Higher nodule number was observed in Arerti than Habru chickpea varieties. The results of this experiment showed variability in nodulation among the varieties, which might be due to inadequate compatibility between the varieties used and strain applied. In conformity with this result Kenani *et al*. (2012), reported that Ethiopian and introduced chickpea germ plasma were high in genetic diversity for both symbiotic and agronomic characters. The two and three interaction effect had not significant effect on number of nodule per plant.

Table 2. Effects of variety, *Rhizobium* strain and phosphorus rate on number of nodule, Nodulating rate, nodule colour, nodule volume and nodule dry weight of Chickpea.

Treatments	Number of Nodule $plant^{-1}$	Nodulation rating $plant^{-1}$	Nodule Colour	Nodule Volume(ml) $plant^{-1}$	Nodule Dry Weight (mg) $plant^{-1}$
Variety					
Arerti	37.79^{a}	6.20	2.32	0.65	136.47
Habru	34.74^{b}	6.08	2.33	0.63	136.09
LSD (5%)	2.382	ns	Ns	ns	Ns
rhizobium inoculation					
Uninoculated	35.29^{b}	5.92^{b}	2.32	0.61^{c}	136.13^{ab}
EAL 018	35.68^{b}	5.96^{b}	2.34	0.64^{b}	132.90^{b}
EAL 029	37.83^{a}	6.56^{a}	2.32	0.67^{a}	139.80^{a}
LSD (5%)	1.371	0.373	Ns	0.029	5.1647
phosphorus kg ha^{-1}					
0	33.64^{c}	4.65^{d}	2.30	0.60^{c}	132.67^{ab}
15	35.29^{b}	5.56^{c}	2.30	0.62^{c}	127.58^{b}
30	36.76^{ab}	6.57^{b}	2.34	0.63^{bc}	138.50^{ab}
45	38.08^{a}	7.08^{a}	2.36	0.68^{a}	141.42^{a}
60	37.42^{a}	6.86^{ab}	2.35	0.67^{ab}	141.22^{a}
LSD (5%)	1.770	0.481	Ns	0.038	6.667
CV (%)	7.31	10.09	4.69	9.05	7.33

Means in column followed by the same letter are not significantly different at 5% level of significance.

Nodulation rating

Nodulation rate was significantly ($P<0.05$) affected by the main effect of *Rhizobium* inoculation and P_2O_5 level. Similarly, interaction effects of variety x inoculation, inoculation x phosphorus, and variety x phosphorus x inoculation .The present result revealed that inoculation with EAL 029 rhizobium strain increased nodulation rate by 10.81% as compared to control. The higher nodulation due to inoculation resulted in higher nitrogen fixation and eventually produced higher number of pods per plant which bring about higher grain yields as a whole (Singh *et al*., 2011). Similarly, significant ($P <0.05$) variation was observed due to P_2O_5 fertilizer application. Nodule rating per plant was increased by 47.5% and 52.3% due to application of 60 kg P_2O_5 and 45 kg P_2O_5 ha^{-1} compared with control treatment. These results are in accordance with the findings of Alemu (2009) who reported that fenugreek varieties fertilized with P_2O_5 alone showed highly significant differences in nodulation rating at 26 kg P_2O_5 ha^{-1}.

Arerti variety chickpea inoculated with strain EAL 029 produced the higher nodule rating (6.75 $plant^{-1}$), followed by Habru variety inoculated with strains EAL-029 (6.37 $plant^{-1}$). Similarly, Arerti variety applied with 45 kg P_2O_5 ha^{-1} provide higher (7.49 $plant^{-1}$) nodulation rate per plant followed by Habru variety with 60 kg P_2O_5 ha^{-1} (7.13) per plant. Nodule rating was also influenced by interaction effect of variety, *rhizobium* inoculation and phosphorus rates. Maximum (8.04 gm $plant^{-1}$) nodulation rate was recorded from variety Arerti when inoculated with EAL 029 and application of 45 kg P_2O_5 ha^{-1} followed by the same variety when inoculated with EAL 018 and applied with 30 kg P_2O_5 ha^{-1} .This result indicates that nodules root colonization was affected by P_2O_5 fertilizer. This is because when nutrients are available to the optimum level, effective and large nodules colonize the tape root systems. Consistent with this suggestion Jennings (2004) reported that, mature, effective (nitrogen fixing) nodules are often clustered on the primary root and have pink to beef stick red centers.

Nodule color

The nodule colour of chickpea varieties in the current finding was non-significantly ($P<0.05$) varied in all treatments. The developed nodule color ranged between pink to slightly dark red for all treatments. This revealed that both the inoculated and the indigenous *Rhizobium* were effective irrespective to chick pea varieties and rates of P_2O_5 application. With this regard, many authors reported that legume nodules having dark pink or red centers due to presence of leg hemoglobin are an indication for effectiveness of the rhizobial strain used and it is correlated to nitrogen fixation (Butler and Evers, 2004).

Nodule volume per plant

Analysis of data indicated that nodule volume per plant was significantly affected by inoculation treatment and phosphorus application rates but not by two and three way interaction effect. Among both strain the mean maximum nodule volume (0.67 ml) was produced by EAL 029 strain inoculation treatment while the lowest (0.61 ml) volume nodule per plant was recorded from control. This means that, inoculation of chickpea varieties with effective bacterial strain increased nodule volume and at the same time it increased effectiveness of nodules. Purohit (2001) stated that, the rate of nitrogen fixation of nodule is directly proportional to the volume of the effective nodule. Nodule volume was significantly ($P< 0.05$) varied because of P_2O_5 fertilizer application. The highest volume (0.68 ml) was produced when 45 kg P_2O_5 ha^{-1} was applied, while the lowest (0.32 ml) nodule volume was recorded from the control. The results are in line with Alemu (2009), who reported that highly significant variations were observed in nodule volume per plant of fenugreek varieties in response to increasing the rate of phosphorus application.

Table 3. Interaction effect of variety and phosphorus application rate on nodulation rating of chickpea varieties.

Variety	P_2O_5 (kg ha^{-1})	Nodulation rating per plant
Arerti	0	4.46^{f}
	15	5.71^{cd}
	30	6.78^{ab}
	45	7.49^{a}
	60	6.58^{b}
Habru	0	4.84^{ef}
	15	5.42^{de}
	30	6.36^{bc}
	45	6.67^{ab}
	60	7.13^{ab}
LSD (5%)	0.821	
CV (%)	14.24	

Means in column followed by the same letter are not significantly different at 5% level of significance

Table 4. Interaction effect of variety and Rhizobium inoculation on nodulation rate and nodule dry weight of chickpea varieties.

Variety	Rhizobium	Nodule rating per plant	Nodule dry weight per plant
	No inoculation	6.15^{ab}	135.53^{ab}
Arerti	EAL 018	5.71^{b}	129.77^{b}
	EAL 029	6.75^{a}	144.10^{a}
Habru	No inoculation	5.68^{b}	136.73^{ab}
	EAL 018	6.2^{ab}	136.03^{ab}
	EAL 029	6.37^{ab}	135.50^{ab}
LSD (5%)		0.902	8.69
CV (%)		20.22	8.79

Means in column followed by the same letter are not significantly different at 5% level of significance

Nodule dry weight per plant

The dry weight of nodule was significantly ($P < 0.01$) affected by the main effect of P_2O_5 rates and rhizobium inoculation. Phosphorus application at 60 and 45 kg P_2O_5 ha^{-1} was significantly increased nodule dry weight by 6.44 and 6.60% respectively as compared to the control. This is probably due to the positive role that P_2O_5 plays in promoting nodulation and enhancement of photosynthesis in plants. This is in accordance with the finding of Amza (2002) who reported that higher phosphorus level resulted in greater dry weight of nodule per plant of chickpea while the lower P_2O_5 level provide minimum dry weight of nodule per plant. Similarly, inoculation of chickpea by EAL 029 rhizobium strain gave the highest (139.80gm per plant) nodule dry weight while the lowest (132.9 mg per plant) nodule dry weight was obtained from control, but significant variation was not observed between varieties on nodule dry weight. The current results are in agreement with previous reports of Assefa (2016) who reported that chickpea varieties inoculated with strain EAL-029 produced higher nodule number, nodule dry weight and shoot nitrogen content followed by strain ICRE-03.Interaction of variety and inoculation revealed that the. The highest (144.10gm $plant^{-1}$) nodule dry weight was recorded from Arerti variety inoculated with EAL 029 while the lowest (129.77 gm $plant^{-1}$) was obtained from EAL 018 of Arerti. However, significant variation was not observed due to other interaction effects.

Table 5. Interaction effects of variety, *Rhizobium* inoculation and phosphorus application rate on nodulation rating of chickpea varieties.

Treatments			
Varieties	Rhizobium inoculation	P_2O_5 (kg ha^{-1})	Nodulation Rate $plant^{-1}$
Arerti	Without inoculation	0	4.10^{d}
		15	6.68^{ab}
		30	6.75^{a-e}
		45	6.87^{a-e}
		60	6.37^{c-h}
	EAL 018	0	4.00^{n}
		15	4.15^{i-n}
		30	$6.00^{d-ı}$
		45	7.57^{ab}
		60	6.85^{a-e}
	EAL 029	0	5.27^{h-m}
		15	6.30^{c-h}
		30	7.60^{ab}
		45	8.04^{a}
		60	6.53^{b-f}
Habru	Without inoculation	0	4.40^{k-n}
		15	5.00^{i-n}
		30	5.40^{f-k}
		45	6.57^{b-f}
		60	7.05^{a-d}
	CP EAL 18	0	5.33^{g-l}
		15	5.85^{e-j}
		30	6.48^{b-g}
		45	6.30^{c-h}
		60	7.03^{a-e}
	CP EAL 29	0	4.80^{j-m}
		15	5.40^{f-k}
		30	7.20^{a-c}
		45	7.15^{a-d}
		60	7.30^{a-c}
Lsd (5%)	1.197		
CV (%)	11.93		

Means in column followed by the same letter are not significantly different at 5% level of significance.

Shoot Nitrogen uptake at 50% flowering

The shoot N uptake of chick pea was significantly ($P<0.05$) varied due to main effect of rhizobium strain and P_2O_5 fertilizer rates. Application of 60 and 45 kg P_2O_5 ha^{-1} resulted in significantly higher N uptake (626.25 and 616.63 mg $plant^{-1}$) respectively while the lowest (563.31mg $plant^{-1}$) N uptake was recorded from the control. *Rhizobium* inoculation with EAL 029 strain significantly increased shoot N uptake at 50% flowering. The highest (630.53mg) N uptake was obtained from inoculation with EAL 029 strain as compared with other treatments. The increased response to all nodulation parameters with increasing levels of Phosphorus in the present study may have contributed to higher N fixation and N uptake at 50% flowering stage. The results are in conformity with those of Hussain *et al*. (2007) who observed maximum N uptake due to increasing rates of P_2O_5 and N up to 60 kg P_2O_5 ha^{-1}. However, significant difference was not observed in N uptake due to variety, and by interaction effect between two and three factors

Shoot phosphorus uptake at 50% flowering

The P uptake was significantly ($P < 0.001$) varied due to individual effect of P_2O_5 fertilizer rate and inoculation with EAL029 strain. As it is evident from the data presented in Table 6, inoculation with EAL 029 strain showed highly significant ($P<0.05$) variation on P uptake as compared to uninoculated treatment. There was no significant difference in P uptake between the two chick pea variety and interaction effect between variety, inoculation and P_2O_5 application rates. Thus, increasing P_2O_5 from 0 to 60 kg P_2O_5 ha^{-1} resulted in about 17.2% increment in P uptake per plant. The increased response of chickpea to applied P_2O_5 in respect to P uptake may be due to the fact that P_2O_5 is essential for plant growth and photosynthesis activity and the high response of rhizobia to applied P_2O_5 were contributed to development of more vegetative growth which in turn increasing more nutrient uptake Addisu (2013). *Rhizobium* inoculation significantly increased shoot P uptake at 50% flowering. The highest (117.82mg) P uptake was obtained from inoculation with EAL 029 strain as compared with other treatments. On the other hand, there was no significant difference in P uptake due to interaction effect.

Table 6. Nutrient uptake of chickpea as influenced by variety, rhizobium inoculation and phosphorus rate.

Treatments	Shoot nitrogen Uptake (mg $plant^{-1}$)	Shoot phosphorus Uptake (mg $plant^{-1}$)
Variety		
Arerti	630.40	116.53
Habru	556.63	102.68
LSD (5%)	Ns	Ns
Rhizobium inoculation		
Uninoculated	578.79^{b}	106.09^{b}
EAL018	576.23^{b}	104.91^{b}
EAL 029	625.53^{a}	117.82^{a}
LSD (5%)	35.22	9.023
phosphorus kg ha^{-1}		
0	563.31^{c}	98.91^{b}
15	574.37^{bc}	108.39^{ab}
30	595.35^{bc}	109.07^{ab}
45	608.30^{ab}	115.79^{a}
60	626.25^{a}	115.87^{a}
LSD (5%)	45.479	11.649
CV (%)	8.02	9.19

Means in column followed by the same letter are not significantly different at 5% level of significance.

Effects on Nutrient Uptake of chickpea varieties

Seed nitrogen uptake

Seed nitrogen uptake was significantly affected by variety, rhizobium inoculation and P_2O_5 fertilizer application. The highest mean N uptake (92.0 and 94.1 kg ha^{-1}) of chickpea seed was obtained from application of EAL 029 rhizobium strain and Arerti variety of chickpea respectively. This result was in agreement with Tahir *et al.* (2009) in which soybean grain, straw and total nitrogen accumulation increased by 9, 122 and 76% over the control as a result of rhizobium inoculation. An increase in N contents due to rhizobium inoculation could be related to significant increase in nodulation resulting in higher accumulation of N through biological N_2 fixation. The main effect of P_2O_5 application showed significant influence on chickpea seed N uptake.

Application of P_2O_5 at the rate of 60 kg P_2O_5 ha^{-1} were resulted in increasing N uptake by 8.8% as compared to the control (0 kg P_2O_5 ha^{-1}). It was significantly increased when the rate changed from 30 to 60 P_2O_5 ha^{-1}. The increase in seed N uptake may be due to the result of increase in plant growth which resulted in high N fixation. Interaction effect of the treatment had no significant effect on chickpea seed N uptake.

Straw Nitrogen uptake

The analysis of variance showed that there was no significant difference in straw N up take of chickpea between variety and interaction effect between applied treatments (variety, inoculation and P_2O_5). However, main effect of inoculation and P_2O_5 highly significantly ($P<0.01$) affected straw N uptake of chickpea. The application of 30 and 45 kg P_2O_5 ha^{-1} increased straw N up take by 13.6 and 18.4% as compared to the rest treatment. The result was similar with Sarawg *et al.* (1999) who reported that application of P_2O_5 increased the shoot N content of chickpea over the control. Similarly inoculation of chickpea with EAL 029 *rhizobium* strain increased straw N uptake by 8.4% as compared to the control.

Total Nitrogen up take

It is evident from data presented that total N uptake was increased significantly with increasing levels of phosphorus. The results showed that total N uptake increased with increasing phosphorus rates, with the highest total N uptake (142.59 and 139.40 kg ha^{-1}) was obtained by phosphorus application 45 and 60 kg P_2O_5 ha^{-1} as compared to the control. Similarly, seed inoculation with EAL 029 *rhizobium* strain significantly enhanced total N uptake of chickpea by 7.95% as compared to the control. The increased total N uptake as the result of increasing P level and inoculation could be due to increased availability of N and P_2O_5 which enhances crop growth. Moreover, there was non-significant difference between chick pea variety and interaction effect of variety and phosphorus, inoculation and phosphorus and three way interactions in total N uptake of chickpea.

Table 7. Grain, straw and total N and P uptake and crude protein content of chickpea as influenced by variety, rhizobium inoculation and phosphorus rate.

Variety	Seed N uptake (kg ha^{-1})	Straw N uptake (kg ha^{-1})	Total N uptake (kg ha^{-1})	Seed P uptake (kg ha^{-1})	Straw P uptake (kg ha^{-1})	Total P uptake (kg ha^{-1})	Crude Protein (%)
Arerti	91.99^{a}	47.86	139.85	14.18	2.57	16.75	20.39^{a}
Habru	87.24^{b}	42.39	129.63	12.69	2.43	15.12	19.87^{b}
LSD (5%)	1.3214	ns	Ns	Ns	ns	ns	ns
Rhizobium Inoculation							
Uninoculated	87.33^{b}	44.11^{b}	131.43^{b}	12.44^{b}	2.39^{b}	14.83^{b}	19.94
EAL018	87.46^{b}	43.45^{b}	130.91^{b}	13.69^{a}	2.30^{b}	15.99^{a}	20.21
EAL 029	94.06^{a}	47.82^{a}	141.88^{a}	14.18^{a}	2.80^{a}	16.98^{a}	20.25
LSD (5%)	4.639	3.6468	7.2986	1.0043	0.3553	1.1353	ns
Phosphorus kg ha^{-1}							
0	85.93^{b}	42.24^{b}	128.17^{b}	12.01^{c}	2.23^{b}	14.24^{d}	19.21^{d}
15	86.35^{b}	41.49^{b}	127.85^{b}	12.32^{bc}	2.28^{b}	14.60^{cd}	19.74^{c}
30	88.57^{ab}	47.12^{a}	135.69^{ab}	13.56^{ab}	2.44^{ab}	16.00^{bc}	19.86^{c}
45	93.47^{a}	49.12^{a}	142.59^{a}	14.78^{a}	2.89^{a}	17.67^{a}	21.22^{a}
60	93.75^{a}	45.66^{ab}	139.40^{a}	14.52^{a}	2.65^{ab}	17.17^{ab}	20.63^{b}
LSD (5%)	5.9889	4.708	9.4225	1.2966	0.4587	1.4656	0.5243
CV (%)	7.99	12.48	8.36	11.54	21.96	10.99	3.11

Means in column followed by the same letter are not significantly different at 5% level of significance; LSD = Least Significant Difference; CV= Coefficient of Variation

Seed phosphorus uptake

The main effects of rhizobium inoculation and P_2O_5 application were highly and significantly (P<0.01) affected chickpea seed phosphorus uptake (SPU). However, interaction effects had no significantly affected on seed P uptake. The results showed that seed P uptake was increased by 23.06 % at 45 kg P_2O_5 ha^{-1} as compared to the control. Havlin *et al*. (1999) also indicated that large quantities of P are found in seed and P_2O_5 is considered to be essential for seed formation. Result of seed phosphorus uptake was influenced by *rhizobium* inoculation. Inoculation by EAL 029 and EAL 018 provide maximum (14.18 and 13.69 kg ha^{-1}) seed phosphorus uptake respectively as compared to uninoculated ones.

Straw phosphorus uptake

Analysis of variance showed that two and three factors (variety, inoculation and phosphorus) did not show interaction effect on straw P up take. Similarly, the main effect of variety was remained non-significant. However, the main effects of inoculation and P_2O_5 level highly significantly (P<0.01) affected chickpea straw P up take. The present result revealed that the variation in straw P uptake in response to P_2O_5 fertilizer application revealed that the soil P_2O_5 levels influence on amount of P uptake by chickpea. Application 45, 60 and 30 kg P_2O_5 ha^{-1} increases straw Phosphorus up take by 29.6, 18.8 and 9.4% respectively as compared to the control. Inoculation with EAL 029 strain show maximum (2.80 kg ha^{-1}) straw P uptake, while inoculation with EAL 018 show minimum (2.39 kg ha^{-1}) straw P uptake.

Total Phosphorus up take

Rhizobium inoculation showed significant (P<0.01) variation on total P uptake as compared to uninoculated treatment. Inoculation of chickpea with EAL 029 and EAL 018 increase total P uptake by 14.50 and 7.82% as compared to the control, respectively. This result was in accordance with Tahir *et al.* (2009) who reported that rhizobium inoculation of soya bean increased total uptake of P by 79%. The higher P uptake due to *rhizobium* inoculation could be due to the fact that some isolates of rhizobia have the ability to solubilize precipitated P_2O_5 components and thereby increase P uptake in plants Qin *et al*. (2011. The main effect of variety and interaction effect had not showed significant effect on total P uptakes at physiological maturity. However, total P uptake was significantly (P<0.05) affected by P application rates. The total P uptake of chickpea was improved by 12.4%, 20.6% and 24.1% at application of 30, 60 and 45 kg P_2O_5 ha^{-1}), respectively as compared to the control.

Amount of N_2 fixed and Percentage of N_2 Derived from the Atmosphere

Rhizobium inoculation and phosphorus application rate significantly (P<0.05) influenced the amount of N_2 fixed and % Ndfa. The mean amount of N_2 fixed and % Ndfa for the application of 45, 60, 30 kg ha^{-1} and inoculation of EAL 029 strain was higher than that of the control (0 kg ha^{-1}) and uninoculated treatment. The result showed that variety and interaction effect of the treatment did not significantly increase the amount of N_2 fixed and the percentage of N_2 derived from the atmosphere (% Ndfa).

The amount of N_2 fixed and % Ndfa increase with increasing phosphorus rate. The maximum amounts (45.69 and 42.96 kg ha^{-1}) of N_2 fixed were recorded from the application of 45 and 60kg P_2O_5 ha^{-1}. Similarly maximum (0.35 and 0.34%) % Ndfa were obtained from application of 45 and 60 kg P_2O_5 ha^{-1}. The amount of N_2 fixed in chickpea was significantly increased by 47.09 and 56.59 %, with phosphorus application of 60 and 45 kg P_2O_5 ha^{-1} respectively over the control (0 kg P_2O_5 ha^{-1}). The %Ndfa increased with the application of 60 and 45 kg P_2O_5 ha^{-1} by 25.93 and 39.63 %, respectively over the control (0 kg P_2O_5 ha^{-1}). The observed improvements with amount of N_2 fixed and % Ndfa increase with increasing phosphorus rate might be due to the P increases the number and size of nodules and the amount of nitrogen assimilated per unit weight of nodules, increasing the percent and total amount of nitrogen in the harvested portion of the host legume and improving the density of Rhizobia bacteria in the soil surrounding the root (Basher *et al*., 2011). This indicates that P deficiency does not only limit plant growth, it can also limit symbiotic N_2 fixation as the latter has been noted to have a higher P requirement for optimal functioning than either plant growth or nitrate assimilation.

Similarly inoculation of chickpea by EAL 029 increase of N_2 fixed and % Ndfa by 35.86 and 18.04% as compared to uninoculated ones respectively. The increase in N_2 fixed and % Ndfa might be due to inoculation of seed with appropriate *Rhizobium* inoculums probably attributed to the enhanced availability of N through BNF for vegetative growth of the plants. Also inoculation of Rhizobium is significantly increase nodule number, nodule weight and root weight (Ali *et al.,* 2008).

Table 8. Effects of variety, inoculation, and phosphorus rate on Post harvest soil, amount of N_2 fixed and percentage of N_2 derived from the atmosphere (% Ndfa).

Treatments	Post harvest Soil N (%)	Amount of N_2 fixed (kg ha^{-1})	% Ndfa
Variety			
Arerti	0.160	40.242	0.33
Habru	0.155	34.226	0.30
LSD (5%)	Ns	Ns	Ns
Rhizobium Inoculation			
Uninoculated	0.151^{b}	32.042^{b}	0.288^{b}
EAL 018	0.157^{b}	36.123^{b}	0.309^{b}
EAL 029	0.165^{a}	43.537^{a}	0.341^{a}
LSD (5%)	0.008	4.790	0.02
Phosphorus kg ha^{-1}			
0	0.148^{b}	29.175^{d}	0.27^{d}
15	0.157^{b}	31.192^{cd}	0.28^{cd}
30	0.153^{ab}	37.202^{bc}	0.31^{bc}
45	0.167^{a}	45.685^{a}	0.35^{a}
60	0.164^{a}	42.915^{ab}	0.34^{ab}
LSD (5%)	0.010	6.183	0.030
CV (%)	7.57	19.86	12.29

Means in column followed by the same letter are not significantly different at 5% level of significance

CONCLUSION

Ethiopia stands first in area and production of chickpea, but third in productivity per unit area after Egypt and Sudan. This clearly indicates the importance of chickpea in Ethiopian agriculture, but requires more efforts to improve its productivity for profitable and sustainable production. The results showed that *Rhizobium* inoculation and application of phosphorus fertilizer rate had significantly differences in nodule number per plant, nodule rating, nodule volume, nodule dry weight, straw N and P uptake at 50% flowering and grain, straw and total N and P uptake at maturity; especially in case of Arerti variety inoculation with EAL 029 strain and application of 45 kg P_2O_5 ha^{-1}. Therefore, proper fertilization program including phosphorus integrated with inoculation of effective rhizobium strain should be implemented to improve the productivity of grain legumes and thereby increase the productivity of legumes and provide good quality chickpea in our Country.

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